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Identification of Prostate Cancer-Specific microDNAs

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14. ABSTRACT

Emerging evidence has suggested that eukaryotic cells can express a special group of extrachromosomal circular DNAs (eccDNAs), called microDNAs. Unlike previously reported eccDNAs, microDNAs are relatively small in length, map to unique DNA sequence, and arise from genes, mostly likely resulting from microdeletions. Since they are usually in a circular form, they are more resistant to exonuclease than linear DNAs and can be stably present in the cells or even possibly in the circulating system. Therefore, overall goal of this application is to determine whether prostate cancer cells express such microDNAs which can be used to serve as valuable biomarkers for prostate cancer diagnosis or prognosis.

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microRNA, lncRNA, biomarker

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Introduction

MicroDNAs are a special group of extrachromosomal circular DNAs (eccDNAs) derived from chromosomal repetitive sequences, intermediates of mobile elements or viral genomes. Unlike most eccRNAs, microDNAs are small, map to unique DNA sequence, and arise from genes, mostly likely resulting from microdeletions. Since they are usually in a circular form, they are resistant to exonuclease and more stable present in the cells or even possibly in the circulating system. We hypothesize that prostate cancer may exploit this mechanism for its own advantage and thus may express a very different microDNA pattern from normal prostate tissue. This different pattern can be detected by currently advanced technology such as deep sequencing. Therefore, overall goal of this application is to determine whether prostate cancer cells express specific microDNAs which may serve a potential biomarkers for prostate cancer diagnosis or prognosis.

Body

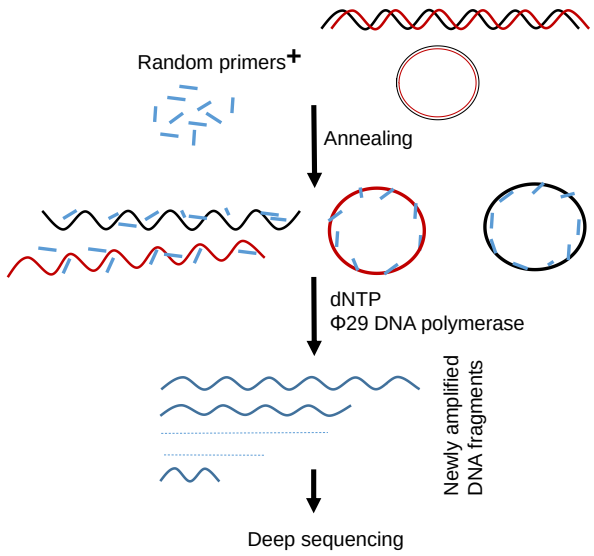

Fig. 1Amplification of DNA by multiple displacement amplification (MDA).

Little is known about microDNAs, and it is not clear whether prostate cancer cells carry potential microDNAs. Thus our goal is to demonstrate the existence of microDNAs in prostate cancer. We

adopted multiple displacement amplification (MDA) with random primers for enriched circular DNA by rolling circle amplification (RCA) (Fig. 1) and then amplified DNA fragments were subject to deep sequencing.

Sequence	NO of Reads
seq 1	184
seq 2	133
seq 3	2407
seq 4	166
seq 5	126
seq 6	221
seq 7	1388
seq 8	108
seq 9	139
seq 10	158
seq 11	968
seq 12	115
seq 13	460
seq 14	391
seq 15	364
seq 16	157
seq 17	634
seq 18	438
seq 19	368
seq 20	529
seq 21	209
seq 22	171
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seq 24	110
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seq 27	109
seq 28	191
seq 29	103
seq 30	188
seq 31	125

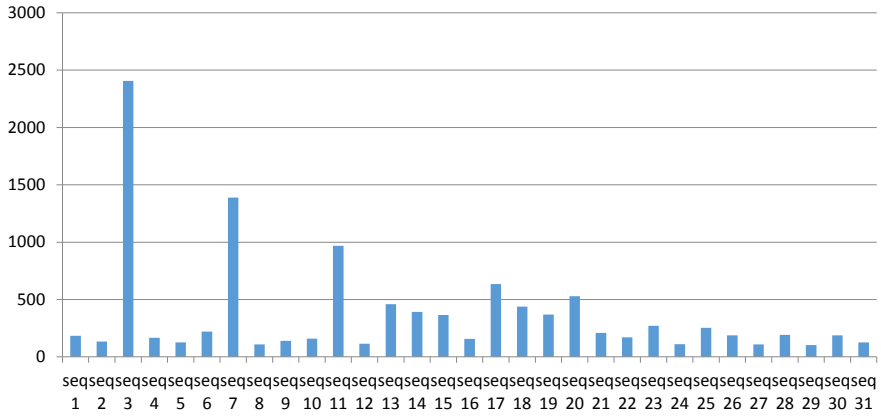

Fig.2 Identification of potential microDNAs from prostate cancer.

>LNCAP2-4
AATCATCRCATAGAATCGAATGMAATTATCATCGAATGGAATCGAATRGAATCAACATCAAACAGAAAAAAACATAATT
ATCCAATTAAATCGAAGATAATCATTAAATGTACCCAAATGCAATCATCTAATGGAATGGAATGGAATAATCCATGGAC
TCGAATGCAATCATCATCGAATGGAATCGAATGGAATCATCGAATGGACTCGAATGGAATAATCATTGAACGGAATCGA
ATGGAATCATCATCGGATGGAAATGAATGGAATCATCATCGAATGGAATCGAATAGAATTATGGAATGAAATCCAGTGT
GATCATCATCGAATGGACCCGAATGGAATCATCATCCAACGGAAGCTAATGGAATCAACATCGAATGAATCAAATGGAA
ACACCATCGAATTGAAACGAATGGAATTATCATGAAATTGAAATGGATGGACTCATCATCGAATGGATTCGAATGGAAT
CATCGAATAAATTGATTGAATCATCATCGAATGGAATCGAATGGTATCATTGAATGGAATCGAATGGAATCATCATC
AGATGGAAATGAATGGAATCGTCATAGAATGGAATCGAATGGATTCATTGAATGGAATCAGATGGAATCATCGAATGGA
CTGGAATGGAATCATTGAATGGACTCGAAAGGGATCATTATTGAATGGAATTGAATGGAATCATGGAATGGTCTCGATT
GGAATCATTATCAAATGGAATCGAATGGAATCACCGAATAGAATCGAATGGAACAATCATCGAATGGAGTCAAATGGAA
TTATCCTCAAATGGAATCGAATGGAATTATCGAATGCAATCGAATGGAATTATCGAATGCAATCGAATAGAATCATCGA
ATGGACTCGAATGGAATCATCGAATGGAATGGAATGGAACAGTCAATGAACTCGAATGGAATCATCATTGAATGGAATC
GAATCGAATGAATTGAATACAAT

971bp

>LNCAP2-7
GTATAATGTGGTGGCCAGGTGCAGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGTGGGCGGATCACGAG
GTCAGGAGATCGAGACCATCCTGGCTAACATGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCCGGGCGTGG
TGGTGGGCACCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGT
GAGCCGAGATCACGCCACTGCACTCCAGCCTGGGTGACAGAGTGAGACA

286 bp

>LNCAP2-9
GTGTGATGTTCCCCGTCCTGTGTACAAGTGTTCTCATTGTTCAATTCCTAACTATGAGTGAGAACATGYAGTGTTTGGT
TTTCTGTCCTTGCGATAGTTTGCTCAGAATGATGGTTTCCAGCTTCATCCATGTCCCTACAAAGGACATGAACTCATCC
TTTTTTATGGCTGCATAGTATTCCATGGTGTATATGTGCCACATTTTCTTAATCCAGTCTATCATTGATGGACATTTGG
GTTGGTTCCAAGTCTTTGCTATTGTGAATAGTGCCGCAATAAACATACGTGTGCATGTGTCTTTATAGCAGCATGATTT
ATAATCCTTTGGGTATATACCCAGTAATGGGATGGCTGGGTCAAATGGTATTTCTAGTTCTAGATCCCTGAGGAATCGC
CACACTGACTTCCACAATGGTTGAACTAGTTTACAGTCCCACCAACAGTGTAAAAGTGTTCCTATTTCTCCACATCCTC
TCCAGCACCTGTTGTTTCCTGACTTTTTAATGATCACCATTCTAACTGGTGTGAGATGGTATCTCATTGTGGTTTTGAT
TTGCATTTCTCTGATGTCCACTGACGGTGAGCATTTTTTTCATGTCTTTTTTGGCTGCATAAATGTCTTCTTTTGAGAAG

710 bp

>LNCAP2-11
GAGATGGAGTCTTGCTCTGTCGCCCAGGCTGGAGTGCAGTGGCGCGATCTCGGCTCACTGCAAGCTCCGCCTCCCGGGT
TCACGCCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCACGCCCGGCTAATTTTTTGTA
TTTTTAGTAGAGACGGGGTTTCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCTGCCTCGGCC
TCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGTGCCCAGCCGAGATGAATTTTTGACAAAAAAATCAATATGGAAT
TTATTTCGTGTTCACTTAATTTAGGACTCAAAATATCTTATTTCAAGAGACCAAGTATATGGCTAATTGAACAGAGGAA

450 bp

Fig. 3 Nucleotide sequences of representative clones.

Deep sequencing of the amplified DNA fragments identified several potential microDNA sequences. Among them, 31 sequences were listed in Fig. 2. The detailed sequences of 4 clones were shown in Fig. 3. All 4 clones were within 1kb in length. Of interest, clone 7 carries AA, AT or TT dinucleotides, a feature of microDNAs; In addition, its GC content is relatively high (58.4%), another feature of microDNAs.

>LNCAP2-7 (286bp)
GTATAATGTGGTGGCCAGGTGCAGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCG
AGGTGGGCGGATCACGAGGTCAGGAGATCGAGACCATCCTGGCTAACATGGTGAAACCCC
GTCTCTACTAAAAATACAAAAAATTAGCCGGGCGTGGTGGTGGGCACCTGTAGTCCCAGCTA
CTCGGGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGC
CGAGATCACGCCACTGCACTCCAGCCTGGGTGACAGAGTGAGACA

Fig. 4 This clone carries multiple AA, AT or TT dinucleotides, a feature of microDNAs, in addition to relatively high GC content.

Identification of such potential microDNAs is critical to the next stage of investigation, i.e., to determine whether such potential microDNAs are present in blood/serum of prostate cancer patients such that they may serve as biomarkers for prostate cancer. Future work will determine whether the potential microDNAs will impact prostate tumor cell growth and invasion. We will also determine whether they are differently present in prostate cancer patients.

Key Research Accomplishments

- We identified several potential microDNAs from prostate cancer cells through multiple displacement amplification.
- Clone #7 is the top candidate which has been cloned in an expression vector and it will be tested in prostate cancer cell lines for its effect on tumor cell growth and invasion
- We will determine whether microDNAs such as clone #7 sequence are present in blood/serum samples of prostate cancer patients and test their potential as biomarkers for prostate cancer.

Reportable Outcomes

Not yet.

Conclusions

Deep sequencing of MDA samples from prostate cancer cells has identified 31 potential microDNA candidates. Clone #7 is a top candidate based on size, dinucleotide repeats and high GC content. Furthermore, we have cloned this sequence. We are currently determining whether ectopic expression of clone #7 will impact prostate tumor cell growth and invasion.